

Root Restriction as a Factor in Photosynthetic Acclimation of Cotton Seedlings Grown in Elevated Carbon Dioxide¹

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ABSTRACT

Interactive effects of root restriction and atmospheric CO₂ enrichment on plant growth, photosynthetic capacity, and carbohydrate partitioning were studied in cotton seedlings (*Gossypium hirsutum* L.) grown for 28 days in three atmospheric CO₂ partial pressures (270, 350, and 650 microbars) and two pot sizes (0.38 and 1.75 liters). Some plants were transplanted from small pots into large pots after 20 days. Reduction of root biomass resulting from growth in small pots was accompanied by decreased shoot biomass and leaf area. When root growth was less restricted, plants exposed to higher CO₂ partial pressures produced more shoot and root biomass than plants exposed to lower levels of CO₂. In small pots, whole plant biomass and leaf area of plants grown in 270 and 350 microbars of CO₂ were not significantly different. Plants grown in small pots in 650 microbars of CO₂ produced greater total biomass than plants grown in 350 microbars, but the dry weight gain was found to be primarily an accumulation of leaf starch. Reduced photosynthetic capacity of plants grown at elevated levels of CO₂ was clearly associated with inadequate rooting volume. Reductions in net photosynthesis were not associated with decreased stomatal conductance. Reduced carboxylation efficiency in response to CO₂ enrichment occurred only when root growth was restricted suggesting that ribulose-1,5-bisphosphate carboxylase/oxygenase activity may be responsive to plant source-sink balance rather than to CO₂ concentration as a single factor. When root-restricted plants were transplanted into large pots, carboxylation efficiency and ribulose-1,5-bisphosphate regeneration capacity increased indicating that acclimation of photosynthesis was reversible. Reductions in photosynthetic capacity as root growth was progressively restricted suggest sink-limited feedback inhibition as a possible mechanism for regulating net photosynthesis of plants grown in elevated CO₂.

Elevated atmospheric CO₂ affects plant growth primarily by increasing net photosynthetic rates through an increase in CO₂ partial pressure at the site of fixation in the chloroplast (26). Responses of plants to long-term exposure of elevated CO₂, however, are not well understood. Net photosynthesis of some species after long-term exposure (weeks, months) to elevated CO₂ is often lower than net photosynthesis after short-term exposure (days, hours) (6, 8, 22, 23, 25, 28, 29).

When photosynthesis was measured at 1000 μ bar CO₂ in *Desmodium paniculatum* after growth in 1000 μ bar CO₂ for 3 to 7 weeks, rates were 33% lower relative to plants grown in 350 μ bar (29). After 3 weeks of growth in 680 μ bar CO₂, net photosynthetic rates of *Eriophorum vaginatum* measured at 680 μ bar decreased 61% relative to plants grown at 340 μ bar (25). Reduced photosynthetic capacity in elevated CO₂ has been found in cotton growing in pots under nitrogen-limited conditions and under conditions of nonlimiting nitrogen (6, 28). On the other hand, cotton plants grown under field conditions at elevated CO₂ maintained higher photosynthetic capacity compared to plants growing at ambient CO₂ levels (21).

It has been established that stomatal conductance of C₃ plants typically decreases at elevated CO₂ concentrations (14). Studies aimed at separating stomatal and biochemical limitations of photosynthesis, however, have concluded that stomatal closure was not responsible for reductions in photosynthetic rates of plants grown under long-term CO₂ enrichment (6, 8, 30). Efforts to understand the physiological nature of the photosynthetic decline in plants exposed to long-term elevated CO₂ have focused on chloroplast damage due to excessive carbohydrate accumulation (6, 29), on feedback inhibition associated with low utilization of photosynthate (6, 8, 22, 23), and on changes in Rubisco activity (20, 22, 30). Starch often accumulates in chloroplasts in response to long-term elevated CO₂ (3, 6, 29). This increase in nonstructural carbohydrate indicates that the plant cannot use photosynthate at the rate at which it is being produced and, when correlated with decreased net photosynthesis, reflects possible feedback effects on the photosynthetic process (1, 11, 15). In extreme cases, chloroplasts have been damaged due to abnormally large starch grains produced when plants were exposed to long-term elevated CO₂ (3, 29). However, photosynthetic capacity was restored and leaf starch levels declined within several days after plants were transferred from elevated CO₂ to ambient concentrations, suggesting that long-term responses may depend on the source-sink balance of the plant (23).

Few experiments have attempted to correlate changes in source-sink balance with reduced net photosynthetic rates of plants grown in elevated CO₂. Reduced photosynthetic response to CO₂ has been found in soybeans that had high source/sink ratios (4, 18). However, manipulations of sink strength in these studies were achieved by removing seed pods (4) or leaves (18), both of which can directly or indirectly

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affect photosynthesis. This study was designed to determine the effects of reduced sink strength on cotton plants grown with long-term CO₂ enrichment without the possible complications of wounding by organ removal. Pot size was used to control root growth, a major metabolic sink for photosynthetically fixed carbon. The time course of photosynthetic capacity and nonstructural carbohydrate accumulation was followed in cotton plants grown in three atmospheric CO₂ partial pressures and two pot sizes to determine the relationship between root restriction and acclimation of photosynthesis to long-term CO₂ enrichment. In addition, plants were transplanted from small pots to large pots to determine if adjustments in photosynthetic capacity were reversible.

MATERIALS AND METHODS

Growth Conditions

Cotton (*Gossypium hirsutum* L. cv Coker 315) was grown from seed in plastic 1.75 and 0.38 L pots ("large" and "small" pots, respectively) in a mixture of gravel and vermiculite (2:1 v/v). A subsample of plants from small pots was transplanted into 1.75 L pots after 20 d of CO₂ treatment ("transplant" pots). All pots were watered to saturation with one-half strength Hoagland solution (7) each morning and with demineralized H₂O each afternoon.

Ten days after germination the plants were moved from a glasshouse into growth chambers in the Duke University Phytotron. Chamber CO₂ partial pressures were automatically monitored and controlled (10) at 270, 350, or 650 μ bar. Plants were grown under a 12 h photo- and thermo-period. PPFD of $1000 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ was provided by a combination of high pressure sodium vapor and metal halide high-intensity discharge lamps. The day/night temperature was 29°C/21°C. RH was approximately 70% during the day.

Growth Measurements

On day 28 of CO₂ treatment, six plants from each treatment were selected at random for determination of biomass and leaf area. Total leaf area per plant was measured with a LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE). Leaves, stems, and roots were separated and dried at 80°C for at least 48 h before measuring biomass dry weights. This harvest was made when plants began to form flower buds.

Gas Exchange Measurements

Gas exchange measurements were made every 4 d during the CO₂ treatment using an open IR gas analysis system, consisting of a temperature- and humidity-controlled cuvette, an ADC series 225 IR gas analyzer (ADC, Huddleston, UK), and General Eastern 1100 dew point hygrometers (General Eastern Inst. Co., Watertown, MA). All photosynthetic measurements were made under saturating irradiance ($1200 \mu\text{mol m}^{-2} \text{s}^{-1}$), at a leaf temperature of $29.0 \pm 0.3^\circ\text{C}$, and with 1.77 ± 0.079 kPa leaf to air vapor pressure deficit. Net photosyn-

thesis, stomatal conductance, and C_i^2 were calculated according to von Caemmerer and Farquhar (26). The youngest fully expanded mainstem leaves from three plants were used for each measurement.

Net assimilation of CO₂ versus the calculated intercellular CO₂ partial pressure (A-Ci curve) (9) was measured on three plants from each treatment at day 4, 16, and 28. Plants transplanted into large pots were measured before transplanting (day 20), 4 d after transplanting (day 24), and 8 d after transplanting (day 28). CE was estimated as the initial slope of an A-Ci curve which was determined by least-squares linear regression (9). A-Ci curves were used to calculate relative stomatal limitation of photosynthesis using the equation

$$\text{RSL} = (1 - A_{\text{max}}/A_0) \times 100$$

where A_{max} = light-saturated net photosynthetic rates measured with C_a in which the plants were grown and A_0 = light-saturated net photosynthetic rates measured with C_a varied as necessary to produce a C_i equal to the CO₂ partial pressure in which the plants were grown (9).

Starch and Sucrose Measurements

Six leaf discs were taken with a circular cork borer (0.65 cm²) from the lamella of the youngest fully expanded leaf from three plants for starch and sucrose analyses once every week during the 4-week period. All samples were taken at 1700 h. Leaf tissue was stored in 3 mL of 80% (v/v) ethanol at -20°C until analyzed.

Leaf discs were ground in 80% ethanol with a Brinkman Polytron Homogenizer (Brinkman Instruments, Westbury, NY), boiled for 5 min in a water bath, and extracted three times with 80% ethanol. The ethanol-insoluble fraction was digested for 1 h with amyloglucosidase (catalog No. A-3042, Sigma Chemical Co., St. Louis, MO) and the glucose released was determined enzymatically (13). The ethanol-soluble fraction was used for sucrose analyses after evaporating the ethanol and resolubilizing in water, following the assay of Kerr *et al.* (13).

Statistical Analyses

Data were tested for normality and met the assumptions of parametric analysis. Two-way analysis of variance was used to test for main effects and interactions of CO₂ and pot size (Statistical Analysis Systems, Cary, NC) on plant growth, biomass allocation, leaf gas exchange, and leaf starch and sucrose. Least Squares Means Test (SAS, Cary, NC) was used for mean separation of the dependent variables. Differences were accepted as significant if probabilities were less than 0.05.

² Abbreviations: C_i , intercellular partial pressure CO₂; RuBP, ribulose-1,5-bisphosphate; A, net assimilation of CO₂; g_s , stomatal conductance; C_a , external partial pressure of CO₂; CE, carboxylation efficiency; A_{650} , net assimilation of CO₂ at C_a of 650 μ bar; RSL, relative stomatal limitation; SLW, specific leaf weight.

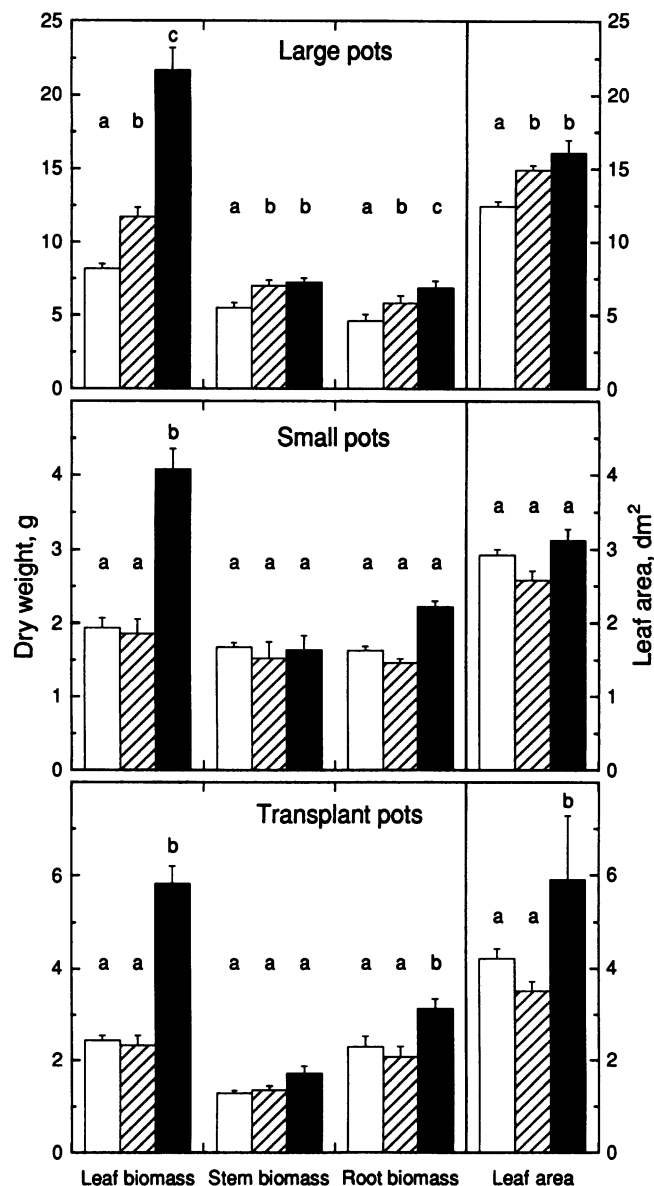


Figure 1. Effects of growth for 28 d in 270 (□), 350 (▨), and 650 μbar CO₂ (■) in large, small, and transplant pots on biomass production, biomass allocation, and leaf area production of cotton. Each bar represents the mean of 6 plants \pm 1 SE. Within pot size treatments, bars which are designated by the same letter are not different at the 0.05 level of significance.

RESULTS

Plant Growth

Root restriction of cotton plants resulted in reduced leaf and stem biomass, as well as reduced root biomass (Fig. 1). On day 28, total plant biomass ($P < 0.0001$) and leaf area ($P < 0.0001$) in all CO₂ treatments were over 250% greater in large pots than in small pots. While pot binding by roots was not quantitatively measured, it was observed that roots filled the area within the small pots and began wrapping around the interior of the pot within the first 8 d of CO₂ treatment.

Root binding also occurred in large pots but at a later date (between day 24 and day 28). Eight days after being transplanted into large pots, plants grown in 650 μbar CO₂ had greater root dry weight (41%) and leaf area (89%) than plants in small pots. Similarly, plants grown in 270 and 350 μbar CO₂ increased root weight (40%) and leaf area (40%) when transplanted into larger pots for 8 d (Fig. 1).

Increasing the CO₂ partial pressure from 350 to 650 μbar significantly increased biomass but not leaf area in large and small pots (Fig. 1). While biomass was much greater in large pots on d 28, the percentage increase in dry weight due to elevated CO₂ was greater in small pots (64%; $P < 0.0245$) than in large pots (46%; $P < 0.0001$). Leaves, stems, and roots responded to CO₂ concentration when grown in large pots, but biomass was not evenly allocated to all plant parts (Fig. 1). Leaf biomass showed the largest response to CO₂. In small pots, leaf biomass was the only plant component to respond significantly to CO₂. At the same time, plants grown in large pots in 350 μbar had greater total dry weight (34%; $P < 0.0001$) and leaf area (20%; $P < 0.0041$) than plants in 270 μbar (Fig. 1). Neither total plant biomass nor leaf area of plants in 270 and 350 μbar CO₂ were significantly different when grown in small pots.

Leaf Starch and Sucrose Concentrations and SLW

A strong effect of growth CO₂ partial pressure was observed on SLW of plants grown in both pot sizes ($P < 0.0001$; Fig. 2). SLW of all plants were positively correlated with leaf starch concentrations ($r^2 = 0.746$; Fig. 2) but showed no correlation with leaf sucrose concentrations ($r^2 = 0.052$; data not shown). Leaves accumulated no sucrose and, in general, the concentration of leaf sucrose remained at low levels in all treatments (below 0.30 mg cm⁻²; data not shown).

In 270 μbar CO₂, plants grown in small pots accumulated greater concentrations of starch than large pots over the 28 d period (Fig. 3; $P < 0.005$). In 350 and 650 μbar CO₂, leaf starch concentrations were not significantly affected by pot size. Eight days after being transplanted into large pots, there was a substantial reduction in leaf starch in all CO₂ concen-

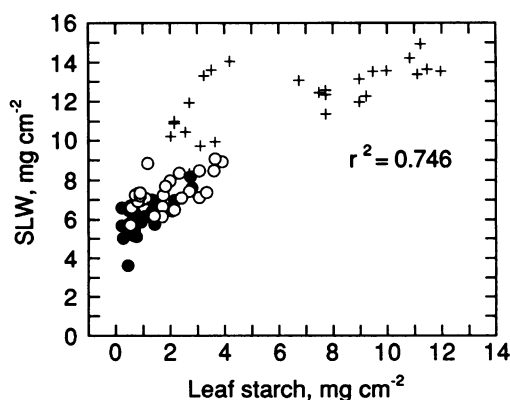


Figure 2. Relationship between SLW and leaf starch of cotton plants grown at 270 (●), 350 (○), and 650 μbar CO₂ (+). Data were collected at 1700 h. $n = 80$.

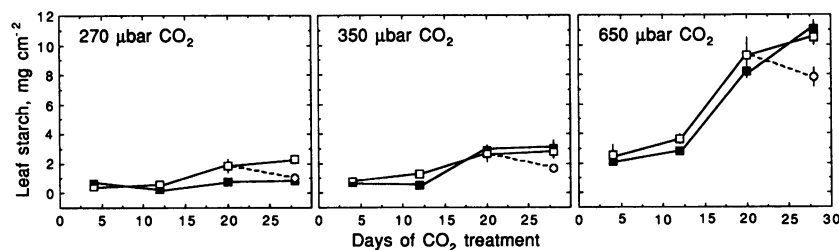


Figure 3. Leaf starch accumulation in cotton grown in large (■), small (□), and transplant pots (○) in 270, 350, and 650 μbar CO₂ over the 28-d period. Data were collected at 1700 h. Each point represents the mean of three replicate measurements. Error bars indicate ± 1 SE but are only visible when they exceed the symbol size.

trations relative to plants remaining in small pots. Transplanted plants grown in large pots had significantly lower leaf starch concentrations than plants grown in small pots in 270 (54%; $P < 0.0129$), 350 (41%; $P < 0.0231$), and 650 μbar CO₂ (25%; $P < 0.0029$).

Plants grown in 650 μbar CO₂ had twice the amount of leaf starch relative to plants in 350 μbar on d 4 in both pot sizes (Fig. 3; $P < 0.0021$). On day 28, leaf starch was almost four times greater in plants grown in 650 μbar than plants in 350 μbar CO₂ ($P < 0.0001$). In large pots, plants grown in 350 μbar CO₂ had greater leaf starch levels than in 270 μbar after 20 d of CO₂ treatment (Fig. 3; $P < 0.007$). On day 28, leaf starch in plants grown in large pots was almost three times greater in 350 μbar than in 270 μbar ($P < 0.0003$). In small pots, leaf starch concentrations in plants grown in 350 and 270 μbar were not significantly different after 28 d of CO₂ treatment.

Leaf Gas Exchange

The time courses of A, g_s, and C_i of cotton plants over the 28 d CO₂ treatment are shown in Figure 4. No significant effects of pot size on A of plants grown in 270 μbar CO₂ were observed (Fig. 4A). In addition, there was no significant change in A over the experimental period in plants grown in

270 μbar in large ($P < 0.129$) or small pots ($P < 0.293$). In 350 μbar CO₂, A was not significantly affected by pot size, but there was a significant decline in rates in large (12%; $P < 0.0025$) and small (16%; $P < 0.0001$) pots between day 4 and day 28. Transplanting had no significant effect on A of plants grown in 270 μbar CO₂, whereas rates of plants grown at 350 μbar were increased by 41% ($P < 0.0039$) after 8 d of being transplanted. Plants maintained higher rates in 350 μbar CO₂ than in 270 μbar throughout the experiment ($P < 0.0001$). On day 28, plants grown and measured at 350 μbar had higher photosynthetic rates in large (18%) and small pots (29%) than plants grown and measured at 270 μbar.

A strong effect of pot size was observed on A of plants grown in 650 μbar CO₂ (Fig. 4A). Plants grown in small pots showed a rapid reduction in A ($P < 0.0001$). Rates declined 15% between day 4 and day 8 and were reduced by 46% by day 28. In contrast, plants grown in large pots in 650 μbar CO₂ showed a much slower decline in A ($P < 0.0001$). There was only a 14% decrease in A between day 4 and day 24. There was a sharp decline in A, however, after 24 d (34%), at which time plants grown in large pots had become obviously pot-bound. Rates increased 69% after plants grown in 650 μbar CO₂ were transplanted into large pots for 8 d ($P < 0.0007$). On day 28, a strong CO₂ \times pot size interaction was observed for A ($P < 0.0099$). Net photosynthetic rates of

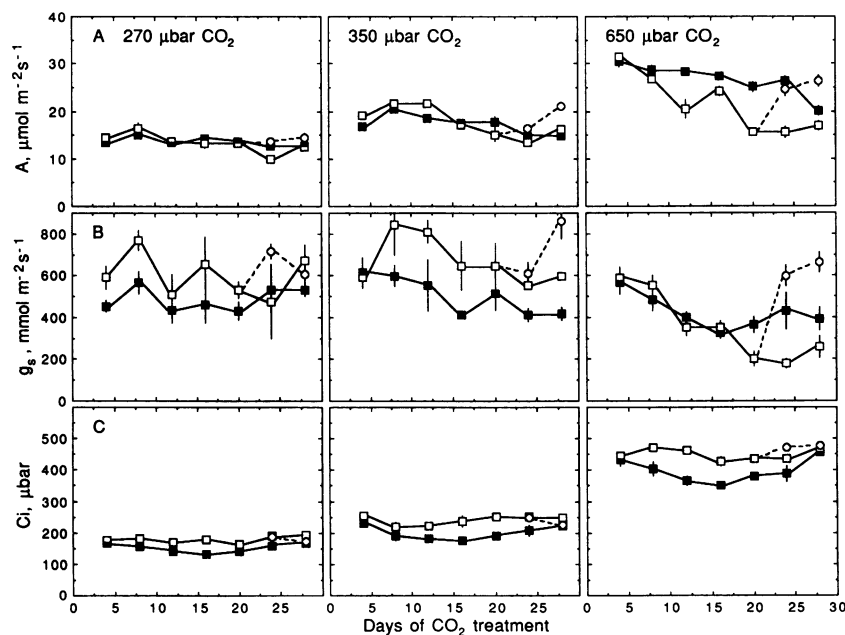


Figure 4. Time course of net photosynthesis (A), stomatal conductance (B), and intercellular CO₂ partial pressure (C) of attached cotton leaves grown in large (■), small (□), and transplant pots (○). Plants were measured under growth conditions at 270, 350, and 650 μbar CO₂. Each point represents the mean of three replicate measurements. Error bars indicate ± 1 SE but are only visible when they exceed the symbol size.

plants in large pots grown and measured at 650 μbar CO₂ were 36% greater than plants grown and measured at 350 μbar . In contrast, rates of plants in small pots grown and measured at 650 μbar were only 5% greater than plants grown and measured at 350 μbar .

Due to the variability in conductance measurements, g_s was not significantly different between plants grown in large and small pots within any CO₂ treatment (Fig. 4B). In 270 and 350 μbar CO₂, however, there was a trend for greater g_s in plants grown in small pots. Over the 28 d period, there were no significant changes in g_s in plants grown in 270 and 350 μbar in large or small pots. In contrast, g_s of plants grown in 650 μbar CO₂ decreased significantly over the 28 d period in large ($P < 0.031$) and small pots ($P < 0.006$) with plants in small pots showing the greater decline. Eight days after transplanting, g_s of plants in 350 ($P < 0.045$) and 650 μbar ($P < 0.0007$) CO₂ increased significantly. Conductance of plants grown in large pots was not affected significantly by growth CO₂ concentration after 28 d. On the other hand, g_s was 57% lower in 650 μbar than in 350 μbar when plants were grown in small pots ($P < 0.0006$).

Except for the measurements on day 4, plants grown in small pots had higher C_i than plants in large pots in all CO₂ treatments ($P < 0.001$; Fig. 4C). On day 28, plants grown in small pots had higher C_i relative to plants grown in large pots in 270 μbar ($P < 0.0378$) and 350 μbar CO₂ ($P < 0.0236$). In contrast, C_i of plants in 650 μbar CO₂ in large pots increased on day 28 when plants were becoming pot bound and were not significantly different from plants grown in small pots. No significant effect of transplanting on C_i was observed in any CO₂ treatment.

CO₂ Response of Photosynthesis

At low C_i , the near linear relationship between A and C_i reflects the capacity of the mesophyll to fix CO₂, *i.e.* CE (26). The upper nonlinear portion of an A- C_i curve reflects the

ability to regenerate RuBP (26). A_{650} was used as a relative measure of RuBP-regenerating capacity because all A- C_i curves did not saturate over the range of CO₂ partial pressures used in these measurements.

A- C_i curves of plants grown in large pots in 270 and 350 μbar CO₂ indicated very little change in the ability to regenerate RuBP over the 28 d period (Fig. 5A; Table I). While CE was not significantly different on day 2 and day 28, there was a significant increase in CE on day 16. In small pots, plants grown in 270 and 350 μbar CO₂ showed slight reductions in CE and the ability to regenerate RuBP by day 28 (Fig. 5B). A- C_i curves of plants grown in 650 μbar CO₂ in large pots indicated no reduction in either CE or the ability to regenerate RuBP between day 4 and day 16 (Fig. 5A). There was a large reduction in both parameters on day 28, however, when the plants were observed to be pot bound. In contrast, plants grown in 650 μbar in small pots showed a quick decline in both the initial slope and upper nonlinear portion of the A- C_i curves (Fig. 5B). In all three CO₂ treatments, there was a significant increase in the ability to regenerate RuBP when plants were transplanted into large pots (Fig. 5C). In addition, CE increased in plants grown in 350 and 650 μbar CO₂ after being transplanted.

Generally, changes in CO₂ response curves over the experimental period did not reflect increased limitations imposed by stomatal conductance (Table I). Percent stomatal limitation of plants within each CO₂ treatment stayed fairly constant regardless of the day of measurement. In 270 and 350 μbar CO₂, RSL increased slightly as plants grown in small pots became potbound. A strong effect of growth CO₂ concentration on RSL was observed throughout the experiment ($P < 0.0001$). In contrast, no effect of pot size was observed.

DISCUSSION

For most C₃ plants, atmospheric CO₂ enrichment produces an increase in net photosynthesis and growth. Some plants,

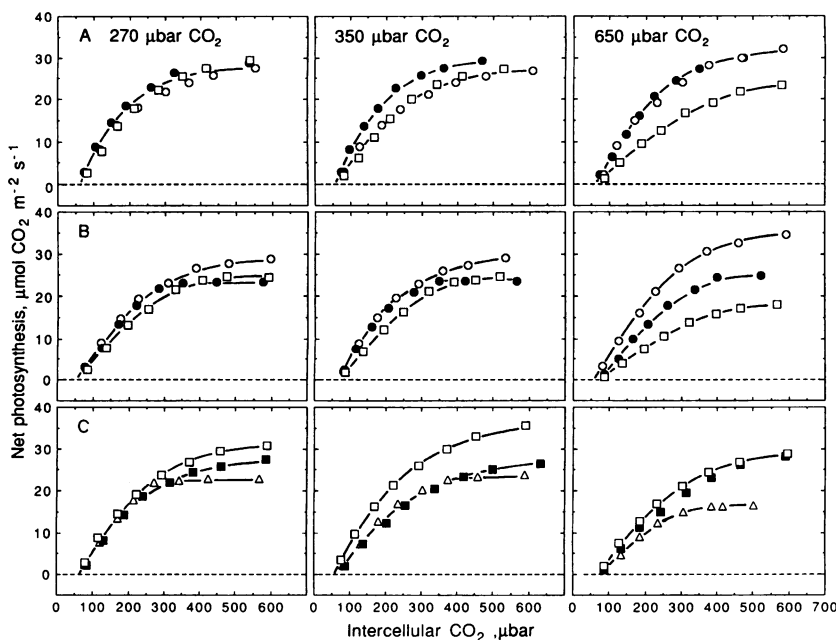


Figure 5. Representative A- C_i curves of cotton plants grown in large (A) and small (B) pots at 4 (○), 16 (●), and 28 d (□) of CO₂ treatment. Transplant pots (C) were measured on the day of repotting (day 20, △), after 4 d (day 24, ■), and after 8 d (day 28, □).

Table 1. Time Course of the Initial Slope of A-Ci Curves (CE), Net Photosynthesis at Ca of 650 μ bar CO₂ (A_{650}), and Percent Relative Stomatal Limitation of Photosynthesis (RSL) of Cotton Plants Grown in Large, Small, and Transplant Pots in 270, 350, and 650 μ bar CO₂.
Each value is the mean of three plants \pm se. Values within any CO₂/pot size treatment which are designated by the same letter are not different at the 0.05 level of significance.

Pot Size	Day	270 μ bar			350 μ bar			650 μ bar		
		CE	A_{650}	RSL	CE	A_{650}	RSL	CE	A_{650}	RSL
Large	4	0.119 \pm 0.008a	25.18 \pm 0.92a	28.6 \pm 0.9a	0.105 \pm 0.002a	25.03 \pm 0.23ab	22.3 \pm 0.7a	0.137 \pm 0.006a	30.47 \pm 1.22a	12.7 \pm 2.1a
	16	0.186 \pm 0.019b	26.88 \pm 0.97a	35.1 \pm 8.3a	0.162 \pm 0.012b	26.95 \pm 1.11a	30.7 \pm 0.5a	0.137 \pm 0.013a	28.30 \pm 0.59a	9.7 \pm 2.2a
	28	0.117 \pm 0.006a	25.67 \pm 0.95a	34.9 \pm 6.4a	0.097 \pm 0.003a	23.28 \pm 1.22b	27.0 \pm 6.0a	0.079 \pm 0.004b	20.19 \pm 0.82b	13.2 \pm 2.0a
Small	4	0.117 \pm 0.001a	26.59 \pm 0.70a	28.9 \pm 1.1a	0.116 \pm 0.009a	27.35 \pm 0.39a	21.1 \pm 2.5a	0.123 \pm 0.005a	31.44 \pm 0.64a	9.0 \pm 2.1a
	16	0.111 \pm 0.007ab	23.33 \pm 3.11ab	29.9 \pm 1.2a	0.111 \pm 0.013a	24.41 \pm 0.56b	25.9 \pm 0.5ab	0.096 \pm 0.006b	24.19 \pm 1.03b	3.0 \pm 0.9a
	28	0.095 \pm 0.005b	23.33 \pm 0.69b	35.5 \pm 0.5b	0.092 \pm 0.002b	24.59 \pm 0.47b	28.3 \pm 0.8b	0.065 \pm 0.002c	16.95 \pm 1.01c	9.1 \pm 4.7a
Transplant	20	0.121 \pm 0.008a	23.40 \pm 0.53a	32.1 \pm 5.3a	0.085 \pm 0.013a	21.47 \pm 2.21a	24.4 \pm 2.4a	0.069 \pm 0.007a	15.67 \pm 0.81a	4.5 \pm 2.4a
	24	0.111 \pm 0.002a	25.56 \pm 0.43a	32.1 \pm 0.6a	0.097 \pm 0.006a	25.14 \pm 0.05a	26.0 \pm 1.0a	0.087 \pm 0.004b	24.53 \pm 1.26b	12.7 \pm 1.4b
	28	0.130 \pm 0.009a	29.28 \pm 1.73b	29.7 \pm 2.3a	0.137 \pm 0.004b	32.59 \pm 0.23b	21.5 \pm 0.1a	0.098 \pm 0.005b	26.41 \pm 1.02b	8.9 \pm 0.8ab

however, exhibit what has been termed "acclimation" to elevated CO₂ partial pressure; photosynthetic capacity is reduced when grown in elevated CO₂ for long periods of time (6, 8, 22, 23, 25, 28, 29). In this study, the decline in photosynthetic capacity that occurred in cotton as a result of growth in 650 μ bar CO₂ was clearly correlated with inadequate rooting volume for the plant. This suggests that reduced photosynthetic capacity of some plants grown in elevated CO₂ in relatively small pots for long periods of time is an artifact of inadequate rooting volume. This interaction may explain why cotton exhibited acclimation to CO₂ in growth chamber studies (6, 23, 28), but not in CO₂-enrichment studies conducted under field conditions (21).

Similarly, the lack of growth response to long-term CO₂ enrichment that has been observed in some studies may have resulted from root restriction. Dry weight gain of cucumbers, grown in 24.5 cm diameter pots, was 83% greater in 1000 μ bar CO₂ than those grown in 350 μ bar CO₂ after 16 d, but no significant CO₂ effect was observed after 43 d (19). Bush bean grown in 0.85 L pots showed no significant CO₂ effects on root growth after 55 d, but a significant interaction between CO₂ concentration and plant age was observed (12). In this study, cotton plants exposed to higher CO₂ concentrations produced more shoot and root biomass than plants grown at lower levels of CO₂ when root growth was less restricted by pot volume. While the percentage increase due to CO₂ enrichment was greater in small pots (64%) than in large pots (46%), results indicate that the dry weight gain in small pots was primarily a consequence of increased specific leaf weight and, therefore, increased leaf starch accumulation. Accumulation of photosynthate in leaves has accounted for most of the dry weight gain due to CO₂ enrichment in studies with soybean (4), bush bean (12), cotton and spurred anoda (17). The results of this study suggest that source/sink imbalance imposed by root restriction severely limited the response to elevated CO₂ and, therefore, are in agreement with the results of studies with soybean in which reduced growth response to CO₂ was found when seed pod/leaf ratio was low (4, 18).

Manifestations of chloroplast damage due to chronic starch accumulation, such as leaf chlorosis, brittleness, and leaf curling (3, 29), were not observed in this study. In addition, stomatal conductance at 650 μ bar CO₂ did not represent a large limitation to carbon assimilation in either pot size. This result was consistent with previously reported effects of elevated CO₂ on stomatal limitations of photosynthesis (6, 8, 30). Reductions in photosynthetic capacity of plants grown in elevated CO₂ have also been attributed to decreased Rubisco activity (20, 22, 30). In cotton, a decline in initial slope of A-Ci curves was associated with decreased rooting volume. When plants had adequate rooting volume, high initial slopes were maintained, suggesting that Rubisco activity was responsive to source-sink balance of the plant rather than CO₂ partial pressure as a single factor. Further evidence of the importance of plant source-sink balance is that reduced Rubisco activity in response to long-term CO₂ enrichment has been observed in older leaves of some plants but not in young or expanding leaves (20, 30). In addition, some studies in which plants were either field-grown or grown in large containers have indicated no effects of growth CO₂ concentration on Rubisco activity (2, 27).

Under conditions of elevated CO₂, in association with

reduced growth rates imposed by root restriction, the reduction in net photosynthesis of cotton conforms to sink-regulated feedback inhibition of photosynthesis, as discussed by Neales and Incoll (15) and Herold (11). This hypothesis is based on the decrease in net photosynthetic rates as root growth was progressively restricted. The decline in photosynthetic capacity was reversed rapidly when sink strength was increased by transplanting plants into larger pots. Leaf starch accumulation in response to CO₂ enrichment also suggests sink-limited carbon metabolism. In this study, however, large concentrations of starch accumulated in leaves of plants grown in 650 μ bar CO₂ regardless of pot size. Starch accumulation before sink demand declined from root restriction indicates that rates of sucrose synthesis or phloem loading also may have limited carbon translocation through the plant. In addition, cotton leaves can maintain high rates of photosynthesis despite accumulating high levels of starch (21).

The mechanism of sink-limited feedback inhibition of photosynthesis is complex and not fully understood. It has been established that Pi availability is a key component in regulation of photosynthesis under certain conditions, such as high atmospheric CO₂ or low temperatures (24). If the rate of CO₂ assimilation (use of Pi) is greater than the use of triose phosphate (release of Pi) in starch and sucrose synthesis, Pi limitation to CO₂ assimilation may be expressed as a reduction in Rubisco activity and RuBP-regenerating capacity and CO₂ assimilation shows a reduced sensitivity to both CO₂ and O₂ concentration (24). In this study, A-Ci curves of cotton with restricted rooting volume indicated that there were reductions in both RuBP-regenerating capacity and CE, despite the CO₂ partial pressure in which the plants were grown. The degree of these reductions, however, was magnified when plants were grown in 650 μ bar CO₂. In addition, the quick reversal in photosynthetic capacity that took place when plants were transplanted into large pots indicates that an "upward" regulation of photosynthesis occurred with the transient increase in growth capacity.

As atmospheric CO₂ increases, source-sink balance of plants may lead to differential responses between species with different growth forms and within species at different stages of their development. Reduced photosynthetic capacity of cotton grown under field conditions in elevated CO₂ was observed in the late developmental stage after fruit maturity (21). While indeterminate species may compensate for increased CO₂ by producing strong sinks such as increased root systems, tillering, or seed production, determinate species may not be genetically capable of increasing the number or size of carbon sinks. CO₂ enrichment resulted in increased growth in the early part of a study with determinate peas, but at maturity no differences in total biomass production were observed (16).

Similarly, environmental restrictions on growth may alter source/sink relationships, thereby reducing the capacity of a plant to respond to a CO₂-enriched atmosphere. Source-sink imbalance imposed by low temperatures and nutrient limitations may explain the reduced CO₂ response in *Eriophorum vaginatum*, a tussock-forming sedge, after one growing season in a field study in upland tundra (25). On the other hand, in nutrient-rich temperate estuaries, large growth responses to CO₂ have been observed in *Scirpus olneyi*, a species that maintains large carbon sinks for extended periods of time (5).

In conclusion, it appears that there is a relationship between

atmospheric CO₂ partial pressure, plant developmental stage, and carbon acquisition and allocation. While many plants respond to CO₂ enrichment by increasing photosynthesis and biomass production, some plants may show reduced responses to CO₂ because of low sink demand imposed by genetic or environmental limitations. Furthermore, studies in which plants are grown without adequate rooting volume may lead to inaccurate conclusions about how plants will respond when grown in elevated CO₂ partial pressures.

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